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NUTRITION REVIEWS

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NUTRITION REVIEWS

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THE NUTRITIVE EVALUATION OF PROTEIN: A HALF-CENTURY OF PROGRESS

The essential nature of protein molecules upon which their nutritional evaluation depends was to a large extent revealed by biochemical studies starting with the identification of glycine as a component of proteins in 1820. By 1903 all but two of the amino acids commonly found in food proteins had been identified (H. B. Vickery and C. L. A. Schmidt, *Chem. Rev.* **9,** *169 (1931)*). Methionine was isolated as a protein component in 1922, and threonine in 1935.

The quantitative determination of the component radicals of proteins naturally proceeded at a slower pace. It involved, first, the preparation of pure proteins from biologic material. The isolation of vegetable proteins by the methods initiated by Ritthausen and Weyl "reached its highest point in the hands of Osborne and his coworkers at the Connecticut Agricultural Experiment Station" (C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins," *C. C. Thomas, Springfield, Ill. (1938)*) in the period from 1890 to 1901. Very little was known of the quantitative occurrence of the monoamino acids in proteins until 1901, when Fischer proposed their separation by the fractional distillation of their esters. The Kossel and Kutscher method for the estimation of the basic amino acids was described in 1900, and the nitrogen-distribution method of Van Slyke was proposed in 1911. These methods have been supplemented from time to time by specific procedures for the chemical estimation of individual amino acids. The wide variety of proteins to which these methods were applied, as well as the incomplete and fragmentary nature of the results secured before 1917, are revealed in a summary published by R. H. A. Plimmer ("The Chemical Constitution of the Proteins," *3d edition, Longmans, Green and Co., London (1917)*).

A significant development in the chemical study of proteins was the application of analytic methods to the mixed proteins of foods, prepared by the extraction of food materials by proper solvents, or by other means to remove interfering substances, particularly carbohydrates. Grindley and his associates (H. S. Grindley, W. E. Joseph, and M. E. Slater, *J. Am. Chem. Soc.* **37,** *1778 (1915)*; T. S. Hamilton, W. B. Nevens, and Grindley, *J. Biol. Chem.* **48,** *249 (1921)*) were among the first to make this application; on a much larger scale and with improved chemical methods, R. J. Block and D. Bolling initiated their investigation of the amino acid contents of foods in 1940 and summarized their results, together with other information secured from the literature, in 1945 ("The Amino Acid Composition of Proteins and Foods," *C. C. Thomas, Springfield, Ill.*).

An intense stimulus to the chemical study of proteins and protein mixtures was the development of microbiologic technics for the quantitative estimation of amino acids. The first application of these methods to amino acids was made by K. A. Kuiken, W. H. Norman, C. M. Lyman, and F. Hale (*Science* **98,** *266 (1943)*), using essentially the same procedures employed by E. E. Snell and F. M. Strong (*Ind. Eng. Chem. (Anal. Ed.)* **11,** *346 (1939)*) to determine riboflavin. In the intervening years many publications on microbiologic (and other) methods (M. S. Dunn, *Advances in Chemistry Series* **3,** *13 (1950)*) for the assay of amino acids and on the results secured with proteins and food protein mixtures have appeared. The compilation of this material in the second edition (1951) of the book by Block and Bolling referred to above, presents an impressive picture of the intense interest in and the available information of

the present time on the amino acid constitution of many food proteins.

While undoubtedly the nutritive value of proteins is largely determined by the proportions of amino acids resulting from their complete hydrolysis, or is limited by such proportions, a mere inspection of tables of amino acid composition affords an uncertain basis for nutritive evaluation. From the diversity in structure of the amino acids commonly found in proteins, biochemists were loath to believe that one amino acid could be converted into another, or could, in general, be synthesized from other dietary constituents, and hence a common opinion was that differences in amino acid constitution necessarily implied differences in nutritive value. This opinion was strengthened by demonstrations with experimental animals, early in the present century, that tryptophan, lysine, cystine, and histidine were indeed indispensable fragments of the protein molecule. The mutual relationship of tyrosine and phenylalanine, and of cystine and methionine were soon suspected. On the other hand, the dispensability of glycine, proline and oxyproline, glutamic acid and aspartic acid was established by 1932 as securely as the methods then available permitted. With the discovery of threonine, the last of the amino acids essential for growth, the division of the proteinogenous amino acids into different categories of nutritional significance was accomplished expeditiously and effectively by W. C. Rose and his colleagues (*Physiol. Rev.* **18,** *109* (*1938*)). The essential amino acids were shown to be threonine, histidine, lysine, tryptophan, phenylalanine, methionine, leucine, isoleucine and valine. The nonessential amino acids were identified as glutamic and aspartic acids, alanine, serine, proline and hydroxyproline. In between these two categories are four amino acids, arginine, glycine, cystine and tyrosine, which Block and Bolling (*J. Am. Dietet. Assn.* **20,** *69* (*1944*)) have aptly called "semi-indispensable," the first two because they can be synthesized by the animal (or avian) body though at rates not adequate for maximum growth, and the last two because they are indispensable only if the food supply contains less than certain minimal amounts of methionine and phenylalanine, respectively.

This classification of the amino acids was an important advance in the nutritive evaluation of proteins by chemical analysis, since attention could now be centered, perhaps exclusively, on the essential amino acids. But even so, the precise evaluation by such means was not at hand. Proteins could be compared by graphing their amino acid "contours" or "spectra," but 1 per cent of one amino acid in a protein obviously possesses a different nutritive significance than 1 per cent of another. What was missing was a yardstick of comparison, represented by the amino acid content of a protein or protein mixture with perfect availability in digestion and metabolism. The mixed proteins of egg conform to this description very closely, a fact established by H. H. Mitchell and G. G. Carman for the growing rat (*J. Biol. Chem.* **68,** *183* (*1926*)), by M. L. Bricker and Mitchell for the mature rat (*J. Nutrition* **34,** *491* (*1947*)), and by E. E. Hawley, J. R. Murlin, E. S. Nasset, and T. A. Szymanski (*Ibid.* **36,** *153* (*1948*)) for adult man.

Proceeding on this basis, H. H. Mitchell and R. J. Block (*J. Biol. Chem.* **163,** *599* (*1946*)) compared the contents of the essential amino acids in single proteins and in the mixed proteins of foods with those of egg, by computing the percentage deviations for each amino acid. The nutritive value of the protein was expressed as a "chemical score," equal to the greatest percentage deficit in an essential amino acid in the protein or protein mixture under scrutiny. This score equals 100 for any protein or protein mixture completely lacking in any one essential amino acid or

it equals zero if the score is taken as 100 minus the greatest percentage deficit, as later proposed by the same authors (*Nutrition Abstr. Rev.* **16,** *249 (1946)*). B. L. Oser (*J. Am. Dietet. Assn.* **27,** *396 (1951)*) has proposed a somewhat similar chemical method of scoring the nutritive value of proteins by the computation of an "essential amino acid index" (EAA index), using egg protein as a standard and computing "egg ratios" (percentages of each amino acid in the protein on the content in egg protein, except that percentages over 100 are considered as 100, and 0 percentage is taken as 1). The EAA index is then the geometric mean of the egg ratios. The two methods of scoring are highly correlated, the correlation coefficient for the 31 values, excluding egg protein, given in Oser's table 8, being 0.95.

The biologic evaluation of proteins may be said to date back to 1872 when C. Voit (*Z. f. Biol.* **8,** *297 (1872)*) showed that gelatin was unable to support tissue growth. The first sustained and productive investigations on this subject in the present century were carried out by T. B. Osborne and L. B. Mendel from 1911 to 1920. Confining attention at first to the vegetable proteins that Osborne had previously isolated and analyzed, Osborne and Mendel distinguished between those proteins that are capable of supporting normal growth in the rat, and those that are not. From such purely qualitative studies, they developed quantitative methods of protein appraisal, involving control of the caloric, as well as the protein, consumption of their experimental animals, culminating in their excellent comparative study of casein, lactalbumin and edestin (*J. Biol. Chem.* **26,** *1 (1916)*). In 1919, with E. L. Ferry, they developed "a method of expressing numerically the growth-promoting value of proteins" (*Ibid.* **37,** *223 (1919)*), involving the determination of the gain in body weight per gram of protein consumed at that level of dietary protein associated with the highest ratio. This method was used in an effective comparison of the proteins of barley, oat, rye and wheat (Osborne and Mendel, *Ibid.* **41,** *275 (1920)*).

The method of Osborne, Mendel and Ferry has been used by many laboratories in this country and abroad for the assessment of the nutritive value of the proteins of foods with modifications designed not to improve the precision of the method, but to increase its convenience at a saving of time. The "protein efficiency ratio" varies from zero to 4.00 when the dietary protein is about 10 per cent and the feeding period four to six weeks, starting with weanling rats.

The nitrogen metabolism method naturally commends itself to the study of protein utilization in nutrition, since by following the wastage of dietary nitrogen in digestion and in metabolism, coefficients of protein utilization pertaining to both can be computed. E. V. McCollum made such a study on growing pigs in 1914 (*J. Biol. Chem.* **19,** *323 (1914)*), in comparisons of the proteins of milk, corn, wheat, and oats; the results were expressed as percentages of ingested nitrogen absorbed (corrected for metabolic fecal nitrogen), and percentages of absorbed nitrogen retained. However, the latter percentages are not true efficiency ratios since they do not credit the absorbed nitrogen with the replacement of endogenous losses of nitrogen, the maintenance requirement. K. Thomas (*Arch. f. Anat. u. Physiol., Physiol. Abt., p. 219 (1909)*) proposed a method of computing a "biological value" of dietary protein in adult human nutrition that avoids this error; the principle of Thomas' method has been adapted to growing animals by H. H. Mitchell (*J. Biol. Chem.* **58,** *873 (1924)*). Both methods are based upon the validity in essence of the theory of protein metabolism proposed by O. Folin (*Am. J. Physiol.* **13,** *117 (1905)*). The principle of the Thomas method has been applied mainly to growing

rats, but also to pigs, sheep, cattle, man, and, with modifications, to poultry. The pig is particularly tolerant of the depletion of dispensable protein stores required in the method (McCollum, *Ibid.* **29,** *215 (1911)*). The biologic values of dietary protein in the growing animal may vary from 0 to 100, but a value less than about 40 seems to be incompatible with growth in the rat. In the pig, McCollum (*loc. cit.*) has observed a remarkably high utilization of the incomplete proteins, gelatin and zein, for the replacement of endogenous losses of nitrogen, equivalent to biologic values computed by the Thomas method of 60 and 80, respectively. This is the first demonstration that the amino acid requirements for nitrogen equilibrium are largely "particulate" in character, as contrasted with the "aggregate" requirements of growth.

The nutritive values of proteins for maintenance have been shown to be different from those for growth, dependent upon differences in amino acid requirements. For growth, casein is definitely superior to edestin, but for maintenance the reverse is true (Osborne and Mendel, *J. Biol. Chem.* **26,** *1 (1916)*); for growth, beef muscle and wheat gluten proteins exhibit biologic values of 76 and 40, respectively, while for maintenance the values are very nearly the same, 69 and 65 (H. H. Mitchell and J. R. Beadles, *J. Nutrition* **40,** *25 (1950)*).

When the essential amino acid contents of proteins or mixtures of proteins in natural food products are accurately known, can their nutritive values be assessed without resort to biologic assay? Can analytic methods displace biologic assays in protein nutrition as they have so largely in vitamin nutrition? We may consider the problem in a restricted area, *i.e.*, the nutrition of the growing rat, upon which so much information is available. By the use of the "chemical score" of Mitchell and Block, or the "essential amino acid index" of Oser, the analytic data on proteins can be reduced to a single value in predicting biologic efficiency; it makes little difference which method of reduction is used, since the two are so highly correlated. They are both highly correlated with the corresponding biologic values, 0.86 and 0.85, respectively. By squaring the mean correlation coefficient, 0.855, the coefficient of determination, 0.73, is obtained, which measures the explained variance in the two variables. In other words, 73 per cent of the variance in each is explained by the demonstrated relationship between amino acid composition and utilization in the anabolism of growth. This means that 27 per cent of the variance is unexplained. Part of this unexplained variance may be traceable to the inaccuracies both in chemical analysis and in biologic assay, although the discrepancies noted in the recent cooperative investigation sponsored by Rutgers University (*Nutrition Reviews* **9,** *56 (1951)*) include some evidently inept performance in some of the cooperating laboratories. However, it may be seriously questioned that this 27 per cent unexplained variance will ever be reduced to an inconsiderable magnitude, because there are many factors in the utilization of dietary protein that are unrelated to the chemical availability of amino acids for anabolic purposes. The following factors may be enumerated: differential rates of enzymic liberation of the amino acids in digestion (*Ibid.* **7,** *269 (1949)*); differential availability of amino acids in digestion (K. A. Kuiken and C. M. Lyman, *J. Nutrition* **36,** *359 (1948)*); differential renal clearances of the amino acids from the blood (L. D. Wright *et al.*, *Am. J. Physiol.* **149,** *130 (1947)*; B. F. Steele, M. S. Reynolds, and C. A. Baumann, *J. Nutrition* **40,** *145 (1950)*); the time relations in the ingestion of different proteins (R. M. Leverton and M. R. Gram, *Ibid.* **39,** *57 (1949)*) and of proteins and carbohydrates (P. S. Larson and I. L. Chaikoff, *Ibid.* **13,** *287 (1937)*; H. N. Munro, *Ibid.* **39,** *375 (1949)*; E. Geiger, *Science* **111,** *594 (1950)*); and the differential sparing effects of carbohydrates and fats in the diet on amino acids

(P. B. Pearson and F. Panzer, *J. Nutrition* **38,** *257 (1949)*; D. Schwimmer and T. H. McGavack, *N. Y. State J. Med.* **48,** *1797 (1948)*). In assessing the nutritive value of the proteins of heated foods, or foods stored over long periods of time, there are indications that the biologic value, or the digestibility, of the contained proteins is impaired before the amino acids themselves undergo chemical changes or destruction. This subject will not be discussed here (*see Nutrition Reviews* **5,** *83 (1947)*; **8,** *193 (1950)*; **9,** *146 (1951)*).

It may be concluded, therefore, that the chemical evaluation of proteins by amino acid analysis will give a first approximation of their value in metabolism, but not in digestion, that it will aid in explaining differences in the metabolic utilization of proteins, and in attaining the most effective supplementation among different protein foods. However, biologic evaluation of proteins and protein mixtures is still the "court of last resort."

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ULTRAVIOLET LIGHT AND EGG PRODUCTION

Egg production and the hatchability of eggs is usually improved when laying hens have access to sunshine. The beneficial effects of sunshine cannot be attributed entirely to its capacity to synthesize vitamin D from provitamin D, a process that requires ultraviolet energy. This has raised the question in the minds of many poultry nutritionists as to whether or not sunshine supplies nutritional factors other than vitamin D that are essential for egg production and hatchability. T. C. Byerly, H. W. Titus, N. R. Ellis, and R. B. Nestler (*Poultry Sci.* **16,** *322 (1937)*) obtained evidence to support such a view. They noted a significantly greater egg production in hens receiving direct sunlight than in hens that were confined, but adequately supplied with vitamin D.

Recently H. G. Barott, L. G. Schoenleber, and L. E. Campbell (*Poultry Sci.* **30,** *409 (1951)*) have investigated the influence of ultraviolet radiation on egg production. The experiments were conducted in a special underground poultry house that permitted complete control of lighting.

The diet used was an all-mash laying diet and scratch feed (half corn–half wheat). The diet was presumably adequate in all nutritional factors and contained both fish meal and meat meal.

From February through July, one group of 55 hens was exposed to bactericidal radiation (ultraviolet rays with 85 per cent of the output at 2587 Å). Another group of 52 hens served as controls. Light exposure for these hens consisted of fluorescent tubes that emitted no measurable radiation below 3600 Å (insignificant amounts of ultraviolet light). This type of lighting was the same for both groups of hens. For the six-month period the hens under bactericidal radiation produced 86 eggs per hen, compared with 74 eggs per hen for the controls, a 16 per cent increase in egg production attributable to bactericidal radiation. The authors decided that this effect could not be attributed to any vitamin D production since the ultraviolet rays that convert provitamin D to vitamin D are in the range of 2750 to 3000 Å units whereas in this experiment the ultraviolet rays were predominantly 2587 Å. Furthermore, the diet was presumably adequate in preformed vitamin D.

However, to test the validity of this assumption further, 4 comparable groups of hens were treated as follows: Groups 1 and 2 received bactericidal radiation. Groups 3

and 4 received visible light only. Groups 2 and 4 also received double the amount of vitamin D present in the basal diet.

During a nine-month period (November to August) the hens receiving only visible light produced an average of 110 and 107 eggs each with normal and double-normal quantities dietary vitamin D respectively. The hens receiving bactericidal light in addition to visible light produced an average of 125 and 120 eggs each with normal and double-normal quantities of vitamin D respectively. These represented 12 to 14 per cent increases in egg production from bactericidal radiation. It was thus established that the increase in egg production was due to some factor or factors other than vitamin D.

The next experiment consisted of determining the effect of various wave lengths of ultraviolet radiation. Four groups of hens were treated as follows: All groups received visible light. Group 1 received 84 per cent of the total ultraviolet radiation at the 2537 A wave length (bactericidal). Group 2 received the same radiation source except that a filter was used to cut out all the bactericidal radiation. Group 3 received ultraviolet radiation mainly of 2967 Å units (provitamin-D activating) and group 4 received ultraviolet radiation between 3200 and 3800 Å units. This range is neither provitamin-D activating nor bactericidal. During the nine-month period (October to July) the group of hens receiving bactericidal radiation (group 1) produced 196 eggs per hen, compared with production levels of 173 to 179 eggs per hen in the remaining groups. Since group 3 received ultraviolet radiation that promoted vitamin D production, this experiment again confirmed the view that the beneficial effects obtained with the bactericidal radiation could not be attributed to vitamin D.

There was no significant reduction in bacterial populations as a result of bactericidal radiation. Neither could any disorders be traced to bacterial infections. The authors therefore felt that the bacteria population under the various treatments had no effect on egg production. The stimulatory effect of bactericidal radiation (2500 to 2600 Å units) upon egg production in hens thus remains unexplained. Vitamin D does not appear to be involved.

A question of immediate interest in connection with experiments of this type is obviously the possible effect of bactericidal radiation on the production of essential nutrients in poultry house litter. The hens in these experiments had access to floor litter. It is well known that poultry house litter can serve as an excellent source of vitamin B_{12} and it is likely that it could also serve as a source of antibiotics that are known to be important in poultry nutrition.

NUTRITION IN CHRONIC DISEASE

A nation can reach pre-eminence and fulfill its destiny only if its people are capable of high productivity and this, in turn, is possible only when the people enjoy good health. Medicine has aided greatly in promoting the national health; one evidence of this fact is the pronounced increase in the life span. It must be recognized, however, that still too frequently life is prolonged into old age without health and happiness, a circumstance directing attention to the common chronic diseases—hypertension, diabetes, atherosclerosis, cancer, arthritis, degenerative diseases of the liver and kidney—whose etiology is not well defined and which continue as a major challenge to the medical profession and the health agencies.

The part which nutrition may play in the control of the chronic diseases has received enough attention up to the present to warrant the conclusion that the relationship

is not a simple one (R. W. Vilter and C. Thompson, *Pub. Health Reports* **66,** *630 (1951)*). The need for close attention to nutrition may actually be "built into" the structure of the individual; because of a peculiar genetic pattern, the metabolic demand for certain nutrients may be accentuated to a point where even a conventional optimal dietary program fails to provide for the exaggerated needs (R. J. Williams, E. Beerstecher, and L. J. Berry, *Lancet* **I,** *287 (1950)*; *Nutrition Reviews* **8,** *257 (1950)*). If, as seems possible, the common degenerative diseases have their origin in such chronic inadequacies, the role of nutrition in their prevention could be a major factor in the national welfare. It is further recognized that through the establishment of food habits, usually at an early age, by delaying the onset and by slowing the course, nutrition can play a more or less direct part in the control of chronic disease.

The death rates for persons over 45 years of age who are 10 to 90 pounds overweight are respectively 8 to 116 per cent greater than for persons in the normal weight range; it has been estimated that 25 to 30 per cent of the adults in the United States are obese. The case against obesity from the point of view of chronic disease is overwhelming: the increased incidence of diabetes mellitus and of cardiovascular-renal disease, the possibility of accentuating hypertension and accelerating the appearance of arteriosclerosis, the tendency to bring on hypertrophic arthritis, the added pain in rheumatoid arthritis and the increased tendency for accidents are points which are emphasized in day-to-day experience with obese patients. Despite claims to the contrary, which may on occasion play a minor part in the causation of obesity, this condition is essentially the result of consuming more food energy than is needed for the particular manner of living. Here, then, is a very direct relation of nutrition to chronic disease. Where chronic, more or less specific, dietary inadequacies exist, the better-known deficiency diseases appear, although severe scurvy, pellagra or beriberi have largely disappeared. On the other hand, due to peculiar food habits or to exaggerated individual requirements, mild deficiency disease may account for much chronic physical and mental ill health. Another instance of the relation between nutrition and chronic illness is well exemplified in the demonstrated beneficial results of good nutritional guidance during pregnancy. The congenital debility in the infant engendered by a faulty diet of the mother during its development *in utero* may well be a predisposing factor to chronic illness in later life.

The physician must not only attempt the prevention of chronic disease, but he must treat it, and in this situation, again, a knowledge of the broader aspects of nutrition comes to his aid. The psychologic and esthetic side of dietetics will play a part. Meals should be small and palatable with supplementary feedings of milk, eggnog and fruit juices to which a small amount of protein hydrolysate may be added, the aim always being to re-establish appetite with the resultant increase in strength and morale. Essentially normal dietary plans can be used with most of these patients provided certain characteristic adjustments are made such as insulin for the diabetic, restriction of sodium for the patient with chronic congestive heart failure, and augmented protein in cases of tuberculosis and hepatic cirrhosis. Nor can the seasons be ignored, for it is well known that in regions of endemic chronic vitamin deficiency, the severity of the disease increases in the spring and fall and the dietary plan, therefore, needs stricter attention at such times.

The present status of our usable knowledge points the way to needed research. More information regarding body composition is needed (*see* R. A. McCance and E. M. Widdowson, *Proc. Roy. Soc.* (*London*)

B **138,** *115 (1951)*; *Nutrition Reviews* **9,** *299 (1951)*; **10,** *14 (1952)*) before the control of obesity can be put on a firm foundation, for it is realized that components other than fat contribute to body weight. Improved micromethods in biochemistry are required to permit more refined diagnoses and hence to detect incipient deficiencies. Fundamental research in cytochemistry and enzyme action will lead to a better understanding of the basic problems of cell growth and tumor development. There is a close relationship between chronic disease and the aging of tissues, and nutrition is of great importance to both. "Aging need not be synonymous with degeneration," and adequate nutrition can be expected to prove the truth of this statement.

ASCORBIC ACID AND THE CONVERSION OF PTEROYLGLUTAMIC ACID TO CITROVORUM FACTOR

It has been demonstrated that certain natural products contain a factor which is required for the growth of *Leuconostoc citrovorum*. This substance possesses many of the metabolic properties of pteroylglutamic acid (PGA), but has certain physical, chemical, and metabolic characteristics which differ from those of PGA. Citrovorum factor is excreted in the urine of experimental animals and human subjects in quantities proportional to the PGA administered. Moreover, the growth factor is synthesized by rat liver slices when PGA is provided as substrate. This conversion is accelerated by addition of ascorbic acid to the medium (*Nutrition Reviews* **8,** *282 (1950)*; **9,** *24 (1951)*). Experimental evidence presented to date would seem to indicate that citrovorum factor is of significant metabolic importance in the group of compounds with folacin activity.

The relationship of PGA to citrovorum factor and the role of ascorbic acid in stimulation of the synthesis of the growth factor have been studied in investigations recently reported by H. P. Broquist, E. L. R. Stokstad, and T. H. Jukes (*J. Lab. Clin. Med.* **38,** *95 (1951)*). The urinary excretion of citrovorum factor has been determined in normal adult human subjects following administration of PGA or citrovorum factor with and without supplements of ascorbic acid. In other facets of the investigation the relationship between ascorbic acid and citrovorum factor has been studied in scorbutic guinea pigs and in homogenates of liver from folacin-deficient rats.

Microbiologic methods of assay were utilized for determination of citrovorum factor and folacin activity. *Leuconostoc citrovorum* was the test organism used for assay of the growth factor. In these determinations a crystalline synthetic form of citrovorum factor, leucovorin, served as a standard (J. A. Brockman *et al.*, *J. Am. Chem. Soc.* **72,** *4325 (1950)*). The test organism in analysis of folacin activity was *Streptococcus faecalis R.*, and the standard in these analyses was PGA.

The 6 subjects who participated in this study were normal adult men. Urine was collected during six-hour periods from each subject. Little variation was found in the urinary excretion of citrovorum factor by these individuals on ordinary diets. Therefore, diets were not controlled during the period of study. The average concentration of citrovorum factor in urine was about 1.0 millimicrogram per milliliter. This compared with a concentration of folacin of from 5.0 to 10.0 millimicrograms per milliliter. It was apparent, therefore, that not all of the folacin activity in the urine was accounted for as citrovorum factor.

When PGA was administered in dosage of 50 mg. orally, from 32.0 to 55.0 micrograms of citrovorum factor were excreted in the urine during the six-hour period. This

indicated that less than 0.1 per cent of the PGA given appeared in the urine as citrovorum factor. The urinary excretion of the growth factor was increased from two to three times when 1 g. ascorbic acid was administered in oral dosage in combination with 50 mg. PGA. Experimental control of the analyses after these large supplements determined that neither PGA nor ascorbic acid in the urine influenced the bacterial assay of citrovorum factor. The indication is clearly drawn, therefore, that ascorbic acid has a role in the biosynthesis of citrovorum factor in human subjects.

The authors next proceeded to study the effect of leucovorin administered orally and intravenously on the urinary excretion of citrovorum factor and folacin. The oral administration of 2.57 mg. leucovorin was followed by excretion of approximately one per cent as citrovorum factor. Simultaneous oral administration of 1.0 g. of ascorbic acid resulted in a decrease to one-half the percentage of citrovorum factor in the urine. In these experiments folacin excretion was double that of citrovorum factor and was similarly decreased when ascorbic acid was administered. Administration of leucovorin intravenously resulted in the urinary excretion of much larger quantities of citrovorum factor. An average of 22 per cent of the vitamin was recovered from the urine under these circumstances and an equivalent quantity of folacin was excreted.

The observations following the oral administration of PGA could be accounted for by relatively slow conversion of PGA to citrovorum factor or by a slow rate of urinary excretion of the growth factor. The latter explanation would not seem to hold, however, since nearly a fourth of the quantity of leucovorin administered intravenously appeared in the urine within six hours. Similarly, explanation was sought for the low level of excretion following oral administration of leucovorin. It was considered that citrovorum factor might be converted to folacin by the acidity of the gastric contents. However, this concept is hardly tenable since the folacin activity recovered from the urine is only double that of the citrovorum factor. It seems more likely that citrovorum factor given by mouth is metabolized to a substance without folacin activity or is relatively poorly absorbed.

In further exploration of the role of ascorbic acid in synthesis of citrovorum factor it was considered that the two compounds might be reciprocally related in animal metabolism and that the growth factor might alleviate the signs of scurvy. To test this hypothesis, guinea pigs were placed on a diet deficient in ascorbic acid. After nine days of depletion the animals were divided into 3 groups and given no supplement, ascorbic acid, or leucovorin. There was no evidence found that leucovorin alleviated the signs of scurvy or delayed death from the deficiency. Determination of the urinary excretion of citrovorum factor in the control group of animals and those supplemented with ascorbic acid failed to demonstrate any difference in rate of excretion on the twenty-first day on the scorbutic diet. Therefore, the results do not indicate that citrovorum factor will replace ascorbic acid in the diet of guinea pigs.

C. A. Nichol and A. D. Welch (*Proc. Soc. Exp. Biol. Med.* **74,** *52 (1950)*) found that ascorbic acid increased significantly the synthesis of citrovorum factor when rat liver slices were incubated with PGA. Broquist, Stokstad and Jukes (*loc. cit.*) undertook similar experiments with like findings. When homogenates of liver from PGA-deficient rats were placed in a medium containing PGA and ascorbic acid, the quantity of citrovorum factor elaborated in two hours of incubation was twelve times that achieved when PGA alone was added. It was of interest that ascorbic acid without added PGA provided greatly increased synthesis of the factor.

Because of the apparent role of ascorbic acid in the biosynthesis of citrovorum factor,

the authors suggest that the adrenals with their relatively great concentration of vitamin C may be the site of or otherwise may be involved in this synthesis. They point out that one of the adrenal hormones, dehydroisoandrosterone, may replace PGA in the growth of certain microorganisms (D. S. Gaines and J. R. Totter, *Proc. Soc. Exp. Biol. Med.* **74,** *558 (1950)*). The suggestion is a pertinent one, but direct experimental evidence for this relationship is lacking.

The experiments discussed in this review provide important information concerning the urinary excretion of citrovorum factor in humans. Moreover, it is demonstrated that the vitamin probably enters most significantly into systems of folacin metabolism when administered parenterally. Further, it is shown that ascorbic acid favorably influences the utilization of PGA and citrovorum factor administered orally. The exact mechanism of ascorbic acid in the biosynthesis of citrovorum factor is not revealed, but further evidence is given of a significant and interesting metabolic relationship between the two vitamins.

NUTRIENT REQUIREMENTS IN RELATION TO BODY SIZE AND BODY FUNCTION

The adequacy of absolute and relative intakes of individual nutrients should be judged in terms of bodily functions which depend critically on the particular nutrient or nutrients. At the present time we possess only incomplete knowledge of the physiologic role of different nutrients in the complex of integrated functions vital to the maintenance of health and life itself. Nevertheless, the problem concerning the extent to which body functions and body size, as indicators of the mass of metabolizing tissues, determine individual nutrient requirements is a challenging one and an attempt to summarize critically the available information is called for. Such a summary was made by H. H. Mitchell (*Scientia* **85,** *165 (1950)*).

Four functions of nutrients in the body were considered: (1) Provision of materials which enter into such structures as the contractile elements of muscle, the conducting nerve fibers, the retaining membranes of the vascular system, and the absorbing mucosa of the intestinal tract. (2) Maintenance of tissue integrity, of the *status quo* of the body tissues undergoing a continual degradation reflected, in part, in the appearance of nitrogenous end products in the urine (endogenous nitrogen catabolism). (3) Construction of new tissue during the period of early growth and in reproductive activity (and lactation) in the female, and growth of the epidermis and its appendages throughout adult life. (4) Liberation of energy for basal cellular activities and for physiologic work such as mechanical work (contraction) in the muscles, osmotic work in the kidneys, and chemical work in the synthesis of tissue constituents from the end products of digestion.

This classification of body functions is not made the basis for a detailed, systematic and quantitative consideration of nutrient requirements, but the author commented that, assuming approximate anatomic homology among animal species, the nutrients entering into the mechanical structure of the organism would be required in proportion to the weight of the body. No comment is made on the nutrients needed to maintain the *status quo* of the body tissues. The demands for nutrients providing materials for the formation of new tissues will depend on the amount of tissue growth or secretion per unit of time and the proportion of the nutrient in the tissue being formed. The growth rates of different animal species vary markedly. While the daily increments in the young rat or pig may correspond to as much as 2 or 3 per cent of its body weight, the growth rate

of a child is about a hundredth of that rate (0.03 per cent or less). Obviously, in the former case the growth requirements determine a large part of the animal's total daily nutrient requirements. The amount of nutrients needed to form a new tissue is many times that needed for the maintenance of an equal amount of the tissue. The requirements for nutritive materials (carbohydrates, fats, and proteins) whose oxidation results in the production of energy and which enter into the oxidative enzyme systems will be proportional to the amount of energy liberated and to the amount of enzymic work to be done.

In the adult animal, four criteria were used as the actual reference points for nutrient requirements: (1) body weight (W), (2) body surface and metabolic body size, $W^{0.75}$, (3) heat (energy) production, and (4) amount of food consumed. The requirement data are summarized in the table.

Most of the tabular values represent averages obtained for several species. Some indication of the scatter, such as the range, would be useful. Even though the author was concerned with *generalized* requirements, more emphasis in the discussion on interspecies differences and individual peculiarities of the metabolism could have prevented some possible misuses of this and other tables of requirements. It has been noted that urinary excretion of thiamine, used widely as a measure of body stores and of the intake of this vitamin, is highly characteristic of the individual (O. Mickelsen, W. O. Caster, and A. Keys, *Proc. Soc. Exp. Biol. Med.* **62,** *254 (1946)*). In normal young men maintained on daily intakes of 1 and 2 mg. of thiamine, respectively, the thiamine excretion showed a two- to three-fold variation for subjects within each group and an overlapping of the two groups. The men tended to retain the relative level of thiamine excretion when the thiamine intakes were reversed. These individual differences could not be explained in terms of fecal excretion of thiamine, physical activity of the subjects, basal metabolic rate, or the body weight. Mitchell did not consider the interindividual variations, but has pointed out some of the existing large interspecies differences. Thus while the guinea pig requires 6 to 7 mg. of ascorbic acid per kilogram of body weight to prevent microscopic lesions, man requires less than one tenth of that amount (about 0.4 mg.) on the same basis. This, of course, indicates that body

THE NUTRIENT REQUIREMENTS OF ADULT ANIMALS

Reference criterion	
Kilograms of body weight	Vitamin A, 5γ Carotene, 30γ Ascorbic acid, 7 mg.[1]
Square meters of body surface	Digestible protein[2], 22.5 g. Dietary calcium[3], 373 mg. Dietary phosphorus[4], 746 mg. Basal energy expenditure, 900 calories Muscular activity, 450 calories
Kilograms of metabolic body size ($W^{0.75}$)	Digestible protein[2], 1.75 g. Dietary calcium[3], 29 mg. Dietary phosphorus[4], 59 mg. Basal energy expenditure, 70 calories Muscular activity, 35 calories
Calories of heat produced	Water[5], 1 ml. Iodine, 40γ
Kilograms of dry, low-fat food consumed	Thiamine, 1 mg. Riboflavin, 1.5 mg. Pyridoxine, 2 mg. Pantothenic acid, 5 mg. Nicotinic acid[6], 10 mg. Cobalt[7], 0.07 mg. Copper[7], 3 to 5 mg.

[1] Applies to guinea pigs only. [2] Assuming a biologic value of 50. [3] Assuming a utilization of dietary calcium of 30 per cent. [4] Assuming a utilization of dietary phosphorus of 30 per cent. Phosphorus may also be required in proportion to the amount of dry food consumed. [5] Exclusive of fecal water wastage. [6] Decreases with increasing protein intake. [7] These values apply to ruminants only.

weight is not a very useful reference point for vitamin C.

Vitamin A requirement is given as five micrograms (21 I. U.) per kilogram of body weight. The British Vitamin A Subcommittee (E. M. Hume and H. A. Krebs, *Med. Res. Council, London, Special Report Series* **264** (*1949*)) arrived at 1300 I. U. as the daily minimum protective dose of vitamin A. With their average subject weight of 64 kg., the "requirement" is almost identical (20 I. U.) with that given by Mitchell. The importance of this coincidence should not be overemphasized. The Recommended Daily Dietary Allowances (*National Research Council Reprint and Circular Series* **129**, (*1948*)) give the value of 5000 I. U. for both adult males (average weight 70 kg.) and females (average weight 56 kg.), with 1000 I. U. added during the second half of pregnancy and 3000 I. U. during lactation, but with no individual adjustments according to weight.

The body surface is an established standard of reference for the basal metabolic rate. In the earlier literature the caloric output was expressed per unit of body weight, leading at times to absurd results—the basal metabolic rate of the mouse is many times that of the horse if compared on the latter basis. Use of the body surface reduces the interspecies variability as well as differences among normal individuals of the same species. Physiologically, it is not the most ideal reference variable, being determined—like the body weight which enters into its calculations—by tissues of different metabolic significance. From the functional point of view, the fat-free body weight and the "active tissue mass" obtained by subtracting weight of the fat and of the extracellular fluid (and bone minerals) from the gross body weight is preferable. When various methods of expressing the basal metabolic rate are used in the study of the responses of the organism to changes in dietary intake such as starvation and refeeding, the results differ and the interpretation of the changes in the basal metabolic rate would also differ depending on the use of the reference standard.

While the use of body surface (and of the "metabolic body size") has at least an empirical justification in connection with basal metabolism, even though the surface area does not directly determine the basal energy expenditure and the square meter of body surface does not have a clear-cut theoretical significance, any attempt—such as is apparently made by Mitchell—to relate unspecified muscular activity to the body surface lacks rationale. The over-all body size, of which the surface area is a good index, including as it does both height and weight, affects different types of work to a different degree, although the differences expressed in relation to the total energy expenditure are not so large as one might expect on first thought. The calculation of energy expenditure involved in physical activity related and unrelated to body size was considered in some detail in the report of a special committee of the Food and Agriculture Organization ("Calorie Requirements," *FAO Nutritional Studies No. 5, Washington, D. C. (1950), see esp. p. 45 ff.*). The energy expended in walking uphill without load is directly related to the body weight, while the energetic cost of carrying an external load is relatively independent of the weight of the body. It may be noted that the committee proposed the formulae $E = 152\ W^{0.73}$ and $E = 123.4\ W^{0.73}$ for calculating the caloric requirements of adult men and women who are similar in age, activity, and environmental temperature to the reference man and the reference woman but differ from them in body size. This, as all other formulae presented in the FAO report, is designed for calculation of caloric requirements of populations, not of single individuals.

Because the amount of protein needed for maintenance is proportional to basal metabolism, the body surface may be used as a reference point of the protein requirements even though the physiologic meaning of such a unit is not very easy to visualize. Yet the fact remains that body surface is some-

what more closely associated with the maintenance requirement for protein than the gross body weight. D. M. Hegsted, A. G. Tsongas, D. B. Abbott, and F. J. Stare (*J. Lab. Clin. Med.* **31,** *261 (1946)*) obtained a coefficient of correlation of 0.61 between nitrogen balance and nitrogen intake per kilogram of body weight, and 0.71 when the nitrogen intake was expressed per square meter of body surface. Physiologically, the "active tissue mass" appears to be the most significant reference point.

The question of calcium and phosphorus requirements and that of proper reference standards for them are open. Mitchell noted that the calcium excretion corresponds to about one per cent, that of phosphorus to approximately 2 per cent of the maintenance protein requirement, a relationship arrived at from a different set of data by H. C. Sherman and his co-workers some thirty years ago (Sherman, *J. Biol. Chem.* **41,** *173 (1920)*; **44,** *21(1920)*). Mitchell computed endogenous calcium, excreted by the rat, rabbit, pig, and man on a nearly calcium-free diet, in terms of milligrams per basal calorie and obtained values varying within a relatively narrow range from 0.121 to 0.164.

In the table it is the water and iodine intake that were referred to the organism's heat production. The water intake is specified as one milliliter for each calorie of heat produced by the human body. In sedentary men this would yield a total daily water allowance of about 2.5 liters. Such an allowance is valid under comfortable environmental conditions. In a hot environment the human body gains heat, in several ways (solar radiation, sky and ground radiation, convection, and conduction). This heat, added to the calories associated with metabolism, must be lost if elevation of the body temperature is to be prevented. The cooling is effected by evaporation (sweating) and demands additional intake of water which is relatively independent of the energy liberated in the body. Physical work in heat may increase the water requirement two to five times. Mitchell drew attention to the fact that in ruminants allowance must be made for the large losses of water in the feces before computing the ratio of water required to heat produced.

The intake of several micronutrients involved in various enzyme systems was related to the amount of food consumed. Digestion and metabolism of absorbed nutrients involves enzymic reactions. Since the enzyme systems undergo degradation, a steady supply of enzyme substrates must be provided in amounts proportional to the nutrients in the metabolism of which they are specifically involved. As a rough measure, the total food intake may be used as reference. The established validity of the requirements for micronutrients presented in the table varies; from thiamine, the average requirement for which has been established for several species and found fairly uniform, to the requirements for cobalt and nickel which apply only to ruminants. Mitchell pointed out that variations from the tabular value of thiamine requirement may be accounted for, in part, by differences in the fat content of the diet and, probably more importantly, by differences in the sensitivity of the criteria employed in assessing thiamine requirements. The latter point is crucial for arguments establishing nutrient needs, together with a precise definition of requirements, allowances, minimum and recommended standards. Without these provisions the argument is likely to be both endless and fruitless.

Mitchell's attempt to provide a rational and systematic frame of reference for the determination of nutrient requirements of adult animals is not, and in the given state of our knowledge, cannot be fully satisfactory. But it has brought together a good deal of what we do know and will serve, it is hoped, as a stimulus to both theoretical considerations and experimental research designed to fill the gaps and to relate more adequately the nutrient intake to body function.

THE RICE DIET IN HYPERTENSION

Even if the rice-fruit diet instituted by W. Kempner in 1944 (*Nutrition Reviews* **6,** *293 (1948)*) does not prove to be of as great therapeutic benefit as originally postulated, the large amount of investigation which has been stimulated by the original work will have contributed greatly to our knowledge of hypertension, especially in its relation to diet. Some of the many studies which have been done have recently been reviewed (*Ibid.* **8,** *304 (1950)*; **9,** *70, 73, 233 (1951)*).

Two new reports of the rice diet in hypertension have appeared from the Massachusetts General Hospital. The first of these, by D. G. Loofbourow, D. Callahan, and R. S. Palmer (*New Engl. J. Med.* **244,** *577 (1951)*), concerns the therapeutic effect of the rice-fruit diet when used in ambulatory patients. The urinary excretion of chloride determined on a random specimen of urine, when these patients came to clinic, was used to estimate the adherence to the diet. The total number of patients instructed in the use of the rice diet was 105. Only 29 per cent were strict adherers. Thirty-six per cent were classed as moderate adherents and 35 per cent were delinquent. Fear seemed to be one of the important forces prompting adherence to the diet. Only 25 per cent of those adhering strictly to the diet had significant improvement in the disease, according to the authors' criteria.

The authors concluded that the rice-fruit diet was not satisfactory for ambulatory patients. The reasons were that it was unpleasant to the patient, expensive because the patient had to return to the doctor frequently, time-consuming to the doctor, and in some instances the diet might even be dangerous. They did not believe that for ambulatory patients any better results were obtained on the whole than with ordinary medical therapy; however, some patients, particularly those severely ill with hypertension, did improve remarkably. The authors are planning an investigation of the treatment of hypertension with a diet which would be far more palatable than the rice-fruit diet, low in sodium, very low in fat, and with higher than usual carbohydrate content unless weight reduction were necessary. This would seem to be a rational choice of therapeutic nutritional regimes, since it seems quite clear that the greatest effect from the rice-fruit diet is due to its low salt content.

J. H. Currens, E. A. S. Reid, E. A. MacLachlan, and F. A. Simeone (*New Engl. J. Med.* **245,** *354 (1951)*) studied the metabolic effects of the rice diet in patients with severe hypertension. Their results did not differ greatly from those previously reviewed (*Nutrition Reviews* **9,** *233 (1951)*). Seven patients were studied in all, with fore-control periods, then the rice diet, and finally periods in which sodium chloride was added. As others have observed, the negative nitrogen balance which is definite early in the rice diet diminishes and almost reaches equilibrium after two to three months. Weight loss was observed in all patients, although it was not marked. There was a fall in basal metabolic rate, although no change in protein-bound iodine was observed.

ALPHA-TOCOPHEROL AND THROMBOEMBOLIC DISEASE

The role of vitamin E in human nutrition or as a therapeutic agent in clinical disease has not as yet been established. New trials of this agent for various conditions, however, continue to appear periodically (*Nutrition Reviews* **7,** *118 (1949)*). Among the most recently suggested uses of this compound is its administration for the prevention of

thromboembolic disease, particularly as it occurs following surgery (J. H. Kay, *Yale J. Biol. Med.* **23,** *515 (1951)*).

In preliminary studies of the coagulation process, J. H. Kay and S. B. Hutton (*Bull. Tulane Med. Fac.* **9,** *122 (1950)*) reported the following findings: (1) Disodium alpha-tocopherol phosphate added to thrombin prevented clot formation when fibrinogen was added to the thrombin. (2) The addition of calcium to the tocopherol-thrombin solution resulted in precipitation of tocopherol and release of thrombin activity in the supernatant fluid. (3) Alpha-tocopherol and fibrinogen when mixed underwent combination as evidenced by changes in the viscosity of the mixture from that of the original solutions. (4) When calcium was added to the tocopherol-fibrinogen combination a precipitate resulted which was greater in amount than that which took place in (2). The supernatant fluid of this latter reaction contained less nitrogen than before precipitation, which indicated that fibrinogen had been precipitated with the tocopherol. The authors also stated in this article that practically no antithrombic activity remained in the supernatant fluid after the precipitation. However, in the later paper (*Yale J. Biol. Med., loc. cit.*), Kay states that subsequent studies have revealed that there is antithrombic activity in the supernatant fluid, minute in quantity, but sufficient to inhibit amounts of thrombin which clot normal plasma. This point is of some importance in the manner in which clinical prophylaxis of intravascular clotting has been undertaken in subsequent investigations.

A second set of studies was then reported by J. H. Kay, S. B. Hutton, and G. N. Weiss (*Bull. Tulane Med. Fac.* **9,** *127 (1950)*). Investigating the properties of accelerator globulin they found the following: (1) Certain preparations of accelerator globulin which showed antithrombic activity demonstrated thrombic activity when precipitation with calcium was carried out. When the precipitate was redissolved with potassium oxalate, extracted with ether and assayed by the method of A. Emmerie and C. Engel (*Rec. d. trav. chim. de Pays-Bas* **58,** *283 (1939)*; *Nutrition Reviews* **8,** *157 (1950)*), tocopherol was found to be present. (2) The antithrombin thus found to be present in the globulin preparation demonstrated ability to combine with fibrinogen as did the alpha-tocopherol phosphate in the studies first reported. (3) A mixture of fibrinogen, antithrombin (from the accelerator globulin), and thrombin in proper amounts yielded a complex which reacted chemically like a compound termed fibrinogen B described by H. Cummine and R. N. Lyons (*Brit. J. Surg.* **35,** *337 (1947–48)*; *Australian J. Exp. Biol. Med. Sci.* **23,** *131 (1945)*). Kay *et al.* concluded that the finding of fibrinogen B in blood of patients disposed to intravascular clotting as reported by Cummine and Lyons represented the presence of small amounts of thrombin which had been inactivated by forming a complex with antithrombin and fibrinogen. In their studies they found fibrinogen B could be produced in samples of normal plasma to which thrombin was added.

From these studies it was reasoned that phlebothrombosis occurs when thrombin enters the circulating blood, perhaps as a result of surgery. In most patients the presence of antithrombin is presumed to inactivate the thrombin. In a few in whom antithrombin levels are low, thrombosis results. Because of the *in vitro* effect of disodium alpha-tocopherol phosphate on thrombin and the finding of tocopherol structure compounds in the accelerator globulin (from alpha-globulin fractions of plasma), it was assumed that alpha-tocopherol might be the antithrombin (J. H. Kay, S. B. Hutton, G. N. Weiss, and A. Ochsner, *Surgery* **28,** *24 (1950)*). Kay (*Yale J. Biol. Med., loc. cit.*) emphasizes that he does not believe that the sodium salt of tocopherol is the active compound *in vivo*, but speculates on the possibility of the calcium salt of tocoph-

erol being responsible since, as mentioned previously, small amounts of the supernatant fluid from tocopherol-calcium-fibrinogen precipitation can inhibit thrombin-induced clotting. Tocopherol could be present in plasma as a water-soluble lipoprotein, the author states, and if this is so, alpha-tocopherol levels could not be assayed following the therapeutic administration of alpha-tocopherol phosphate unless the tocopherol in blood were hydrolyzed. Because of this he is unable to conclude that the administration of alpha-tocopherol phosphate reproduces a normal physiologic process when it is used as an anticoagulant agent.

In undertaking clinical studies of patients, Kay and his colleagues reported two methods for determination of plasma antithrombin, one involving serial dilutions of plasma to which thrombin and then fibrinogen were added, and the other using serial dilutions of thrombin added to plasma. Initially these were thought to give readings which were identical (*Surgery, loc. cit.*). Later experience with commercial thrombin, however, revealed poor correlation between the two methods because of inability to obtain low antithrombin readings with the latter method. This finding, when thrombin was serially diluted, suggested that other substances in the thrombin solution were also being affected by dilution. In view of this discrepancy it is doubtful that the test measures only the antithrombic content of plasma, and the term "prethrombotic index" has now been suggested instead of "antithrombin determination" (*Yale J. Biol. Med., loc. cit.*).

With this background, clinical studies of the occurrence of antithrombin were undertaken in patients during the postoperative period (J. H. Kay *et al., Surgery, loc. cit.*). Of 120 patients studied, 53 had "antithrombin levels" of 1:16 or less at some time. Thirty of these simultaneously had prothrombin times of twenty seconds or longer (normal fifteen seconds). Twenty-three patients had antithrombin levels of 1:8 or less coincidentally with prothrombin times of less than twenty seconds, and 13 of these developed clinical evidence of thrombosis or embolism. Only one of the 30 with low antithrombin levels and high prothrombin times developed thrombosis. No thromboembolic complications were noted in the 67 patients with levels above 1:16.

In another series of studies, 4 patients with thrombophlebitis received 300 I. U. of alpha-tocopherol daily orally, and a rise in the antithrombin levels from 1:8 or 1:16 to 1:64 occurred. No data to indicate the time over which this occurred or what spontaneous variations in levels had been noted in untreated patients were given. Disappearance of edema and inflammation were observed in all 4 patients, however, during therapy.

Another group of 99 postoperative patients received 200 I. U. of alpha-tocopherol acetate every eight hours. Seventeen patients showed antithrombin levels of 1:16 or less, and 3 developed thromboembolic complications. Because of the lack of statistical difference in the numbers of 1:16 or lower levels in the treated series as compared to the 120 untreated cases, 10 per cent calcium gluconate, 10 cc. intravenously every twenty-four hours, was added to the regimen. In the first 50 cases so treated, no thromboembolic complications were noted, and antithrombin levels could be maintained above 1:16.

Following this early report of clinical trials of alpha-tocopherol, essentially similar results have been reported by this group of workers on two occasions (A. Ochsner, M. E. DeBakey, and P. T. DeCamp, *J. Am. Med. Assn.* **144,** *831 (1950)*; Ochsner *et. al. Ann. Surg.* **131,** *652 (1950)*). Therapy in these studies appears to have been standardized by the use of alpha-tocopherol orally in doses of 200 units every eight hours, unless the patients are unable to take medication by mouth, in which case 100 mg. of alpha-tocopherol phosphate is given intramuscularly

every eight hours. Calcium gluconate intravenously is given daily with either regimen.

In a recent report of the clinical trials of the use of alpha-tocopherol and calcium in the above manner Kay (*Yale J. Biol. Med., loc. cit.*) has summarized their data. Two hundred and forty-six patients who were untreated revealed 23 cases of thromboembolism, of whom 16 were diagnosed clinically. All patients with phlebothrombosis were found to have levels of 1:8 or less in their "prethrombotic index," and normal prothrombin times. No figure, however, is given for the total number of patients with levels in this range. One patient with a higher than 1:8 level developed an embolism. Four hundred and fifty-seven patients received treatment; of these, 5 revealed thromboembolic complications and 15 had 1:8 levels. All with thromboembolism were found to have 1:8 levels or less and no complications were seen in patients with higher levels. No gross differences in the type of operative procedure performed in the 246 control and 395 treated patients is apparent.

This series of studies is of interest to nutritionists and clinicians alike because of the apparent conclusion that vitamin E or related tocopherol compounds are involved in preventing intravascular coagulation in the human. It is worthy of note, however, that the *in vitro* studies may not demonstrate the true process as it occurs *in vivo*. At the present time it has not been demonstrated that there is a change in the blood tocopherol levels in postoperative patients, and, more importantly, no data have been presented that demonstrate changes in tocopherol levels after administration of the various therapeutic agents or that conclusively prove that the "prethrombotic index" rises as a result of therapy. The rationale of the use of calcium is also not completely convincing in view of the fact that *in vitro* studies demonstrated the ability of calcium to precipitate both antithrombin alone and antithrombin in combination with fibrinogen. Although the supernatant fluid from the latter precipitation process is reported to contain antithrombic activity, this activity has not been shown to be due to the presence of alpha-tocopherol.

The method of compiling data is also open to question from a number of viewpoints. Although the small series of patients receiving tocopherol with and without calcium appear to favor the use of calcium concurrently, no statistics are available on the possible beneficial effect of calcium alone. More significantly, it is apparent that the control series and the tocopherol-calcium treated patients were not obtained by treating alternate patients during the same period of time. Such a method would appear to be eminently desirable in this type of investigation. The series of control patients reported shows a 10 per cent (23 of 246) incidence of thromboembolism which would appear to be extremely high for Charity Hospital in New Orleans where these studies were carried out. The frequency of thromboembolism at Charity Hospital for the period from 1948 to 1949 was 0.23 per cent of all cases admitted. In the period 1938 to 1949, 34 per cent of all patients were admitted to the surgical service on which 37 per cent of the thromboembolism cases were seen, indicating no significantly higher occurrence of thromboembolism on this service than in the rest of the hospital population. It would appear, therefore, that even allowing for the number of surgical patients who were not operated upon, the usual incidence of thromboembolism would be under 1 per cent (*J. Am. Med. Assn., loc. cit.*). This is in marked contrast to the 10 per cent incidence in the control series in these studies, but is more consistent with the 1 per cent incidence in the treated series (5 in 457).

The change in interpretation of the test from one determining "antithrombin levels" to that of a "prethrombotic index" also leads to some confusion. It is not clear that all

patients were studied with the first or more reliable method, or whether some patients in the series were studied only with the simpler second method. It is also unfortunate that the total incidence of 1:8 levels in the control series in relation to the number of thromboembolism patients is not stated definitely for comparison with that of the treated series.

If these studies, in spite of the defects mentioned, should reveal the recommended therapy to be a truly effective method of preventing thromboembolic disease, the mechanism of action of vitamin E still remains unclear. If antithrombic activity is a pharmacologic effect it would appear that a definite hemorrhagic diathesis would be produced in at least some patients. If alpha-tocopherol is part of a physiologic process of inhibiting coagulation, such has not been unequivocally demonstrated by this work, and a number of problems in vitamin E physiology in man have yet to be answered.

STABILITY OF VITAMIN A IN FEEDS

The protection of vitamin A against autoxidation when concentrates of this vitamin are added to feeds has been a major problem to feed manufacturers. Stabilizing media, such as oils or other solvents fortified with antioxidants, are excellent for stabilizing vitamin A in solution. However, when such solutions are added to mixed feeds the problem of preventing autoxidation of the vitamin is a difficult one. In efforts to solve this problem the commercial suppliers of vitamin A products have made available dry vitamin A preparations that are claimed to be stable when mixed into animal feeds.

M. J. Burns and F. W. Quackenbush (*Ind. Eng. Chem.* **43,** *1592 (1951)*) have investigated the stability of commercial dry vitamin A preparations. The seven preparations studied ranged in potency from 150 to 1500 micrograms of vitamin A or carotene per gram. Each of the preparations was subjected to a series of 4 storage conditions as follows: stored as received; mixed with ground whole yellow corn; mixed with soybean oil meal (expeller process); mixed with glucose (cerelose). Each of the mixing agents was ground to pass through a 20-mesh sieve before use. The final mixtures contained approximately 10 micrograms per gram of vitamin A alcohol or its equivalent in esters or in beta-carotene. All samples were stored in screw cap bottles in the dark at room temperature and analyzed for vitamin A at monthly intervals for a period of six months.

The most stable preparation contained a mixture of natural antioxidants, while the least stable product contained only one antioxidant and a synergist (sorbital and butylated hydroxyanisole). After six months' storage the most stable product still contained 86 per cent of the initial vitamin A potency when stored without diluent; 75 per cent when stored in corn meal, 81 per cent when stored in soybean meal, and 61 per cent when stored in glucose. After a similar period of storage the least stable product contained 67 per cent of the initial potency when stored without diluent; 46, 55, and 16 per cent when stored in corn meal, soybean meal and glucose respectively.

In general, soybean meal and corn meal as diluents afforded much better protection than glucose, due evidently to the presence of natural antioxidants that supplemented the antioxidants present in the vitamin A concentrates.

The products that were the most stable when stored alone were in general also the most stable when stored in the diluting agents. From the results it is apparent that all of the dry vitamin A concentrates tested represent an improvement over vitamin A

sources that have been used extensively in the past in poultry and livestock feeds.

The relative stabilities of vitamin A emulsions and feeding oils were studied by G. R. Halpern, B. March, and J. Biely (*Poultry Sci.* **28,** *168 (1949)*). Although experimental conditions were not strictly comparable, the data obtained in these earlier studies indicated that vitamin A emulsions and feeding oils are less stable when mixed into feeds than the dry preparations used in the studies by Burns and Quackenbush.

Halpern and associates found that approximately 50 per cent of the vitamin A potency was lost when unstabilized feeding oils were mixed into poultry feeds and stored for five months. It was further noted that stabilization of the oils or emulsions with antioxidants and synergists had very little influence on the vitamin A stability once these preparations were mixed into the poultry feeds. These workers concluded that the antioxidants in vitamin A oil or emulsions lose their effectiveness when mixed into feeds because of the extensive dilution.

The problem of stabilizing vitamin A in mixed feeds is complex because of the many factors that can promote oxidation. Examples are the hydroperoxides and decomposition products derived from fats present in fish and meat meal. Minerals, particularly manganese, which is used extensively in poultry feeds, promote vitamin A oxidation. Halpern and associates noted that vitamin A oil in a mixed feed containing fish meal lost 18 per cent of its potency after eighteen weeks of storage, while the vitamin A oil mixed with the fish meal alone lost over 70 per cent of the vitamin A potency after a similar storage period.

The recent trends toward the use of more vegetable protein sources and vitamin B_{12}-antibiotic concentrates in mixed feeds in place of animal proteins should in general result in feed mixtures with better natural antioxidant qualities and improved vitamin A stability.

The recent results of Burns and Quackenbush emphasize that although progress has been made the problem of stabilizing vitamin A in mixed feeds is still a real one.

VITAMIN B_{12} ANTAGONISTS

To the investigator, an antimetabolite (antagonist) is an extremely useful research tool. Experimental diets deficient in a particular vitamin or amino acid are not always easy to devise. This is especially true for the newer vitamins. The study of the metabolic functions of the vitamins is greatly enhanced, particularly in isolated systems, by the availability of an appropriate antimetabolite. Furthermore, antimetabolites of the vitamins have found useful places as therapeutic agents in certain clinical conditions. Therefore, with the discovery of each new vitamin the nutritionist, the biochemist and the physician have learned to look forward to the discovery of an appropriate metabolic antagonist.

Encouraging results are now beginning to appear in the field of vitamin B_{12} antagonists. 1,2-dimethyl-4,5-diaminobenzene has been identified in vitamin B_{12} as part of a dimethylbenzimidazole riboside. D. W. Woolley (*J. Exp. Med.* **93,** *13 (1951)*) postulated that this compound was probably a precursor of vitamin B_{12} as well as a precursor of riboflavin since it occurs in riboflavin fused to a pyrimidine ring and a ribityl side chain. 1,2-dichloro-4,5-diaminobenzene was then chosen as an antimetabolite and tested in a series of 26 species of bacteria. It was found that this diamine was toxic to those species which did not exhibit a nutritional need for riboflavin plus vitamin B_{12}. In other words, growth of these species which synthesized their own riboflavin and vitamin B_{12} was retarded. The growth-inhibiting action under these circumstances was overcome competitively by the dimethylamino-

benzene, and also, to a lesser extent, by o-phenylenediamine. The toxicity was not influenced significantly by addition of riboflavin plus vitamin B_{12}.

Many structural relatives of dimethyldiaminobenzene were synthesized (D. W. Woolley and A. Pringle, *Fed. Proc.* **10,** *272 (1951)*), several of them for the first time, and tested for potency against *Staphylococcus aureus*, and mice. Alkylation of one amino group, or its replacement by a nitro group, abolished activity even when one or both of the methyl groups was simultaneously exchanged for chlorine atoms. When one hydroxyl replaced one amino group, active agents were formed, either when the methyls were untouched, or when they were exchanged for chlorine. Alkylation of the hydroxyl groups abolished activity. The nitrophenols formed by replacing the amino groups with hydroxyl and nitro groups were very potent, provided that at least one of the methyl groups was also exchanged for halogen. The activity of the aminophenols was only partially reversed by the metabolite, and that of the nitrophenols not at all. Halogenation increased the tendency to noncompetitive behavior with the metabolite; nevertheless, the amino- and nitrophenols still actively inhibited the biosynthesis of vitamin B_{12}.

J. M. Beiler, J. N. Moss, and G. J. Martin (*Science* **114,** *122 (1951)*), in the course of an investigation of the effect of various chemical procedures on vitamin B_{12}, found that treatment of the vitamin in strong acid solution with hydrogen peroxide caused a decolorization of the solution, thus indicating that the cyanide-cobalt complex was attached. This unidentified derivative of vitamin B_{12} was then assayed on *Lactobacillus leichmannii*. It was found that the derivation exerted a marked inhibitory effect on growth, and that this inhibitory effect could be counteracted by vitamin B_{12}. Only at the highest level of inhibitor tested was there incomplete counteraction. No inhibitory effect was observed when this vitamin B_{12} derivative was tested on organisms that did not require preformed vitamin B_{12} as a growth factor.

It will be interesting to follow further biologic work with these antagonists. To date, neurologic lesions, macrocytic anemia, and megaloblastic bone marrow have not been observed in animals made deficient in vitamin B_{12}. Since patients with pernicious anemia exhibit these manifestations and respond dramatically to vitamin B_{12}, it will be of interest to determine if such manifestations appear in animals given a vitamin B_{12} antagonist.

APPARENT INTESTINAL SYNTHESIS OF CAROTENE

W. A. McGillivray at the University of New Zealand has recently presented evidence to indicate that there may be an appreciable synthesis of carotene in the cecum of sheep (*Brit. J. Nutrition* **5,** *223 (1951)*). In digestion trials, designed to obtain an indication of the effect of the composition of feed on the availability of carotene, it was noted that some sheep were excreting, via the feces, more carotene than they were consuming. In the 4 pasture-fed sheep used in the study, the carotene excreted in the feces was 78, 95.2, 97.4, and 114.5 per cent, respectively, of the amount consumed.

In order to gain further information about the fate of carotene in the digestive tract, samples of ingesta were collected immediately after slaughter from the 4 stomachs, from various points along the small intestine, cecum, colon and rectum of each of the 4 sheep. These samples were analyzed for carotene and also for lignin, a relatively inert pasture constituent which was verified to be 96 per cent nondigestible.

The carotene:lignin ratios (milligrams of carotene divided by grams of lignin) of these samples, as well as of the feces, were then determined. Since the lignin is resistant to digestive action, it was assumed that any decrease in the carotene:lignin ratio would be an indication of either carotene decomposition or absorption, and that any increase in the ratio would indicate carotene synthesis in the digestive tract. The average carotene:lignin ratios in the pasture herbage were 18.5. These ratios were fairly well maintained in the samples of ingesta taken from the stomachs, which was interpreted to indicate that there was very little decomposition or absorption of carotene in the stomach.

The carotene:lignin ratio reached a minimum of between 10 and 15 in the mid-region of the small intestine. This was considered an indication of carotene absorption in the upper region of the small intestine. The ratio then gradually increased through the ileum and reached a maximum in the cecum, where the carotene:lignin ratios were between 22 and 28. This increase in carotene, relative to lignin, was interpreted as indicative of carotene synthesis. Some decrease in the ratio was noted in the colon and rectal samples which was assumed to be due to the oxidative destruction of the carotene. The carotene:lignin ratios in the feces were noted to be essentially the same as those of the herbage fed. Thus the over-all result would indicate either that little or no carotene was absorbed, or that some synthesis of carotene had occurred in the digestive tract.

The possibility was considered that the ingesta and the feces might contain yellow pigments, other than carotene, that could have chromatographic properties similar to that of carotene. The absorption spectra of carotene isolated from the herbage and that from the cecal contents were compared and found to be identical, indicating that there were no other yellow pigments.

If intestinal microorganisms are capable of synthesizing carotene in appreciable quantities, it should be possible to demonstrate increases in carotene by incubating cecal contents. McGillivray was not successful in demonstrating such increases. However, when a solid medium (containing agar, tryptose, dextrose and sodium chloride) was inoculated with cecal contents collected under aseptic conditions, carotene was formed in quantities equivalent to 1.2 and 1.8 micrograms per milliliter of medium. No attempt was made to identify the active microorganisms.

A point of immediate interest is whether or not carotene synthesized in the lower regions of the digestive tract can be utilized by the animal. E. R. Barrick, F. N. Andrews, and J. F. Bullard (*J. Animal Sci.* **7**, *539 (1948)*) have reported that no absorption of carotene occurs in the cecum or colon. However, there might be absorption from the lower regions of the ileum, the regions in which the New Zealand report indicates that carotene synthesis may occur.

It is of further interest that C. H. Whitnah, W. J. Peterson, F. W. Atkeson, and H. W. Cave (*J. Agr. Res.* **58**, *343 (1939)*) reported that carotene excretions exceeded carotene intake in animals, an indication that carotene synthesis had occurred. Their results have in general not been widely accepted because they used phasic methods for separating the carotenes. Such methods are not considered specific for the separation of active carotenes from inert yellow pigments.

Although much has been written concerning the role of intestinal microorganisms in the synthesis of various members of the vitamin B-complex, the possible synthesis of a fat-soluble provitamin, such as carotene, has rarely been considered. McGillivray has presented strong evidence that carotenes may be synthesized by intestinal organisms in sheep. The importance of this synthetic source of carotene to the host remains to be determined.

PATHWAYS FOR TRANSPORT OF FATTY ACIDS

Lymph has long been recognized as the principal pathway for the transport of the usual dietary fat. Some of the quantitative aspects of this problem have been discussed (*Nutrition Reviews* **8,** *300* (*1950*)) in a review of an article by B. Bloom and co-workers (*J. Biol. Chem.* **184,** *1* (*1950*)). These workers had noted the appearance of C^{14} in the lipids of thoracic duct lymph, intestinal lymph, and liver following the intragastric administration of palmitic acid labeled with C^{14} in the carboxylic group. The palmitic acid was given either as the free acid or as tripalmitin. Approximately 70 to 90 per cent of the absorbed palmitic acid-C^{14} appeared in the thoracic duct lymph in one group of animals, and about 70 to 85 per cent in the intestinal lymph in another group.

These data were interpreted as corroborating older observations and conclusions that approximately two-thirds of the fat absorbed from the gut is transported in the lymph. However, it was pointed out that since recovery in the lymph of the absorbed fatty acid was incomplete, a small proportion is probably transported by some other route, perhaps the portal blood. However, very small amounts of C^{14}-labeled lipids were found in the livers of these rats, and it was suggested that this might be taken as an argument against portal transport. Further quantitative data regarding the transport routes of fat have now been obtained by B. Bloom, I. L. Chaikoff, and W. O. Reinhardt (*Am. J. Physiol.* **166,** *451* (*1951*)) utilizing C^{14}-labeled fatty acids of different chain lengths.

After cannulation of intestinal lymphatics, rats were fed by stomach tube approximately 8 mg. of a single labeled fatty acid in 0.5 cc. of corn oil. The rats were etherized lightly only during the introduction of the stomach tube. Fifteen to twenty-four hours after the test meal, the rats were sacrificed and samples of the gastrointestinal contents and feces obtained. Absorption values were determined by the differences between administered C^{14} and the C^{14} found in the lipids of the gastrointestinal contents and feces. The C^{14} contents of the lipids of liver and the intestinal lymph were determined.

About 35 to 55 per cent of administered stearic acid-1-C^{14} was absorbed while 80 to 90 per cent of myristic acid-1-C^{14} was absorbed. This is in keeping with earlier observations (*Nutrition Reviews* **5,** *155* (*1947*)) of the relatively poor absorption and digestibility of stearic acid. Approximately 60 to 95 per cent of the fatty acids absorbed were recovered in the intestinal lymph. About 0.1 to 1.5 per cent of absorbed C^{14} was recovered in liver as fatty acid-C^{14}. These figures are similar to those obtained in the earlier work with palmitic acid.

In contrast, when lauric acid-1-C^{14} or decanoic acid-1-C^{14} were fed, almost all of the fatty acids were apparently absorbed. However, only 15 to 55 per cent of the absorbed lauric acid and only 7 to 19 per cent of the decanoic acid appeared in the lymph. About 0.5 per cent of the absorbed C^{14} was recovered in liver as fatty acid-C^{14}.

The possibility that the low C^{14} content of the intestinal lymph of the rats fed lauric or decanoic acids might be due to the loss of fatty acids by bacterial action in the gastrointestinal tract was considered by Bloom *et al.* Accordingly, 2 rats were studied after the number of organisms in the feces was reduced by the feeding of streptomycin and succinylsulfathiazole. The data regarding the absorption of decanoic acid and the appearance of the fatty acid-C^{14} in the intestinal lymph were essentially the same in these animals as in the untreated rats. The authors conclude that bacterial destruction of the fed fatty acid does not explain the low fatty acid-C^{14} recoveries in the lymph of the rats fed lauric and decanoic acids. They suggest, instead, that a large part of the absorbed lauric acid, and almost all the absorbed decanoic acid, enters the portal

blood, rather than the intestinal lymph. This is consonant with the findings of R. H. Hughes and E. J. Wimmer (*J. Biol. Chem.* **108,** *141 (1935)*). They found no butyric acid in thoracic duct lymph of animals which had received butter fat, vegetable shortening, or tributyrin, and concluded that butyric acid passes directly into the portal blood stream.

B. Bloom, I. L. Chaikoff, W. O. Reinhardt, and W. G. Dauben (*J. Biol. Chem.* **189,** *261 (1951)*) have demonstrated that the fatty acids in lymph are present almost entirely as glycerides. It is generally assumed that the conversion of absorbed fatty acids to glycerides occurs in the mucosal cells. Presumably the glycerides cannot traverse the capillary membrane but, instead, enter the central lacteals. In contrast, certain fatty acids can presumably enter the portal blood as such or as phosphatides. Some phosphatides enter the lymph as well. Bloom and co-workers suggest that the differences in the amounts of absorbed fatty acids of varying chain lengths found in intestinal lymph may be related to the capacity of the mucosal cells to incorporate the fatty acids into glycerides, *i.e.*, a short-chain fatty acid is less readily incorporated into glycerides than a long-chain acid. It would appear that another possibility is that the short-chain fatty acids enter the portal blood much more rapidly than long-chain acids, and consequently the former are not so available for glyceride formation as the latter. Direct measurements of the lipid fractions of portal blood would be helpful.

EXPERIMENTAL DIETS AND CANCER

A considerable body of experimental evidence has been accumulated which indicates that in the experimental animal there is a close relationship between particular dietary factors and certain types of cancer. This is particularly true of cancer of the stomach and liver. This important subject has recently been reviewed by K. Sugiura (*J. Nutrition* **44,** *345 (1951)*).

The Japanese workers T. Sasaki and T. Yoshida discovered in 1935 that by feeding o-aminoazotoluene to rats for three hundred days, cancer of the liver could be produced. Shortly thereafter R. Kinosita made the observation that liver cancer could be produced in a shorter period of time by feeding para-dimethylaminoazobenzene, butter-yellow, a pigment used widely to color oils, oleomargarine and other vegetable fat substitutes. These original observations of the Japanese have since been confirmed and extended by many investigators.

When butter-yellow is fed to rats receiving a diet of unpolished rice supplemented with carrots, cirrhosis of the liver generally appears in about sixty days. By ninety days most of the livers of the test animals show cholangiomas (bile duct tumors), and at this time some hepatomas (liver cell tumors) are also present. Then, as time goes on, the production of hepatomas increases. Ordinarily, by the one hundred and fiftieth day the livers of all rats are the sites of cancerous processes involving both cholangiomas and hepatomas. If the butter-yellow is given for less than thirty days no cirrhosis or cancer develops. However, if the feeding of para-dimethylaminoazobenzene exceeds sixty days, liver cancer subsequently develops in about 80 per cent of the animals surviving on the basal diet for a period of sixty days or more after withdrawal of the carcinogenic agent.

The incidence of liver cancer in animals maintained on the unpolished rice-carrot basal diet supplemented with varying amounts of brewers' yeast has been studied, and the results are exceedingly interesting. The addition of 15 per cent of yeast to the rice had a distinct protective effect against the pathologic changes leading to the development of liver cancer. All of the yeast-

supplemented animals examined between one hundred and two hundred days after the beginning of the administration of the diet plus butter-yellow had smooth and practically normal livers, grossly and microscopically. On the other hand, all of the rats fed the butter-yellow rice diet without the addition of yeast and examined on the one hundred and fiftieth days showed typical liver cancer. The inhibitory effect of yeast feeding upon the production of liver cancer was distinctly diminished when the diet contained less than 15 per cent yeast. With the addition of 6 per cent yeast, 40 per cent of the animals had normal livers, 30 per cent had cirrhotic livers, and 30 per cent had livers with a few tumor nodules, when examined on the one hundred and fiftieth day. With the addition of 3 per cent yeast, 30 per cent of the animals showed no malignant changes in the livers and 70 per cent had livers with cancer nodules.

A similar experiment was made with dried beef liver. The results were found to be in general agreement with those obtained with yeast. Thus, it seems evident that both yeast and dried beef liver contain a substance or substances which are inhibitory to the development of liver cancer produced by feeding para-dimethylaminoazobenzene.

Rice is poor in vitamins and low in protein. Therefore, in order to determine whether the protective effect of yeast and liver is due to the correction of a specific dietary deficiency or deficiencies, the effect of various vitamins with or without the administration of casein has been investigated. The daily oral administration of 20 micrograms of riboflavin and 2 g. of casein, which was sufficient to promote normal growth in young rats, was without a significant protective effect. However, when the daily consumption of riboflavin was increased to 200 micrograms, the protective effect was striking, though not complete. From a liver cancer incidence of 70 to 80 per cent, when either riboflavin or casein alone was employed, the figure decreased to 3 per cent. Other compounds, either members of or closely associated with the B-complex group of vitamins, or the combination of cystine and choline, exerted a similar protective effect. Experiments have also shown that the production of liver cancer under these experimental circumstances is definitely reduced by the oral administration of other foodstuffs such as dried kidney, dried spleen, wheat, rye, millet seed, milk and hydrogenated coconut oil. However, a combination of casein and riboflavin, or a diet containing brewers' yeast or dried beef liver is more effective than these other foodstuffs. Surprisingly, biotin, rice bran concentrate, corn oil and commercial hydrogenated cottonseed oil exert a procarcinogenic effect.

The protection against liver cancer formation by yeast or liver is apparently temporary rather than permanent. Rats maintained on a butter-yellow rice diet and 15 per cent yeast for two hundred and eighty to three hundred and fifty days had a 50 per cent incidence of liver cancer. The incidence of cancer after four hundred to seven hundred days was 75 per cent and at eight hundred days was 85 per cent.

If the preliminary feeding with butter-yellow exceeds eighty-five days, liver cancer develops in 100 per cent of the cases, even though dietary anticarcinogenic yeast, liver, or milk is fed for long periods of time after the butter-yellow ingestion. Thus, once adenomatous hyperplasia of the bile duct, cholangioma, or hepatoma is established in the liver these benign and malignant tumors cannot be successfully treated with yeast, liver, or milk.

In summary, these experiments illustrate clearly that various dietary factors affect the development of experimentally induced liver cancer. Certain food elements prevent tumor growth, whereas others stimulate such growth. However, once a cancerous condition has been established in the liver it is impossible to re-establish a normal physiologic state by means of a diet which would

prevent or greatly delay the formation of liver cancer.

It must be admitted that the factors concerned in the development of cancer cells resulting from a combination of dietary deficiency and a carcinogenic agent, are poorly or not at all understood. Likewise, the mechanism by which good diet prevents or delays the development of such experimentally induced cancer is not understood.

The significance of these studies in relation to the development of primary hepatomas in man is not known. However, it is extremely interesting that the incidence of such tumors in well-nourished populations is extremely low, whereas in poorly fed, rice-, corn-, and millet-eating peoples of the world the incidence of such tumors is relatively high (*Nutrition Reviews* **9,** *225 (1951)*).

THERAPY OF ACUTE LEUKEMIA

Acute leukemia is a proliferative disease of leukocytes which is invariably fatal. Until the last few years no specific therapeutic agent or procedure was available which was capable of significantly altering the course of this rapidly fatal disease.

The subject of the chemotherapy of acute leukemia with antimetabolites of pteroylglutamic acid was reviewed in this journal (*Nutrition Reviews* **9,** *143 (1951)*). It was concluded that the results were far from ideal and that although in a certain proportion of patients a partial, temporary remission followed the administration of such an antagonist, cures of this disease had not been obtained.

Recently considerable interest has been centered around the use of ACTH (adrenocorticotropic hormone) and cortisone (compound E) in this condition. Rather enthusiastic reports were given by many investigators at the Third Annual Blood Club meeting held on April 30, 1950 (*Blood* **5,** *785 (1950)*). Recently M. C. Rosenthal and co-workers have reported in detail the results of their studies with these hormones (*Blood* **6,** *804 (1951)*).

A total of 22 patients with acute leukemia were so treated. Of the 15 patients with acute and subacute lymphoblastic leukemia, 2 died before even five days of treatment had been given. Of the 13 remaining cases adequately treated, 9 experienced remissions of brief duration ranging from one to ten weeks. Thus the remission rate was approximately 70 per cent. In children the immediate and over-all remission rate was much higher than that obtained with aminopterin. In the 7 patients with acute myeloblastic or acute monoblastic leukemia, ACTH proved to be of little value and even seemed to intensify the process in some cases.

The authors summarized their results in the following general terms: (1) ACTH and cortisone are active and highly interesting therapeutic agents. (2) Much of the clinical improvement with these hormones relates to their "nonspecific" effects. (3) In acute leukemia, real or objective improvement is transitory at best and may be said therefore to be of "pathetic benefit" since death always occurs although it is a little delayed.

Thus, the second recent promising approach to the therapy of this disease now appears disappointing. This, it would seem, is sufficient cause for researchers in this field to pause and reflect on the questions of why so little has been accomplished in the therapy of this condition, and what newer approaches might be fruitful.

The approach to this problem has been mainly that of therapeutically testing various substances, new as well as old, in men as well as mice. One explanation for the failure to date of this "hit-or-miss" empirical type of approach would seem to be that "good fortune" has not been present. The

success of a continuation of this type of approach must then reside not in an organized scheme, but rather in chance.

Another logical explanation of why so little has been accomplished in the therapy of acute leukemia is that so little is known about the production, distribution, metabolism and ultimate fate of leukocytes. This view has been very clearly stated by Sir Lionel Whitby in an article entitled "Whither Clinical Pathology? Trends and Opportunities" (*J. Clin. Path.* **4,** *129 (1951)*). To quote: "These facts make it abundantly clear that we know little or nothing of the physiology of leukopoiesis, and I am convinced that we shall not understand leukemia until some of these basic physiologic facts have been determined. If half the time and energy which have been devoted to devising and exploiting empirical remedies for the symptomatic treatment of the disease, reducing the leukocyte count, had been expended on basic physiology and pathology, we might well be much nearer to rational treatment."

It is readily apparent that in the normal human body there are influences which are capable of maintaining the circulating level of leukocytes within rather narrow limits. Under particular circumstances, as for example infection, these controlling mechanisms are capable of adjustment so as to allow more rapid production, distribution and utilization of a particular cell type. Knowledge concerning these normal homeostatic mechanisms is extremely scant. One must have information concerning normal leukopoiesis before it is possible to visualize clearly the pathogenesis of a disease where leukopoiesis is abnormal.

W. N. Valentine (*Blood* **6,** *845 (1951)*) has recently reviewed existing information on the biochemistry of leukocytes. The few investigations which have been made into this previously unexplored field have been most interesting. It is already apparent that morphologically identical leukocytes may present substantially different metabolic patterns under varying physiologic and pathologic circumstances.

These patterns in some instances appear highly characteristic of the disease process. For example, unit cell histamine is markedly increased in chronic myelocytic leukemia and greatly reduced in neutrophilic leukocytosis and leukemoid reactions, while the converse is true of alkaline phosphatase activity. Similar differences have been described for other metabolic parameters.

Certainly the study of normal leukocyte synthesis, metabolism and ultimate degradation presents a real challenge for the future. Without such fundamental research, difficult and slow as it is, it is doubtful that a real understanding of leukemia can be obtained. Shorts cuts, as illustrated so well in the past, are likely to be doomed to failure.

RIBOFLAVIN EXCRETION AND NITROGEN BALANCE

Riboflavin occurs in animal tissues associated with protein, and the tissue content of the vitamin has been shown to vary with protein intake (*Nutrition Reviews* **6,** *167 (1948)*; **8,** *133 (1950)*). Also, the riboflavin-deficient animal is not so active in its production of antibodies to injected antigens as are control animals (*Ibid.* **6,** *87 (1948)*). With trauma and burns the requirement for riboflavin as well as for ascorbic acid, thiamine, and niacin is increased (*Ibid.* **6,** *116 (1948)*).

H. Pollack and J. J. Bookman (*J. Lab. Clin. Med.* **38,** *561 (1951)*), in the course of metabolic investigations, have attempted to relate the urinary excretion of riboflavin to nitrogen excretion in patients with a variety of illnesses during periods of positive and negative nitrogen balance. They have observed that increased excretion or decreased

retention of the vitamin occurs when subjects are in negative nitrogen balance.

The patients studied were in the hospital. Food ingested was accurately weighed and the nutrient intake calculated from standard tables of food composition. All urine excreted was collected in twenty-four-hour specimens. Urine specimens were pooled to allow for three-day metabolic periods. Aliquots were taken to determine the content of nitrogen and riboflavin. Fecal nitrogen was not determined, but nitrogen balance was calculated by assuming a constant fecal excretion of nitrogen of 1.3 g. per day.

In a control group of 8 patients who are described only as being well-nourished it was found that the per cent of ingested riboflavin which was excreted in the urine was inversely related to nitrogen balance. With greater retention of food nitrogen there was greater retention of riboflavin. With but few exceptions, patients who were in negative nitrogen balance lost more than 50 per cent of the riboflavin contained in the food by urinary excretion. The diet consumed by these subjects was a hospital diet affording an average intake of 2070 calories, 13 g. nitrogen and 2.1 mg. riboflavin per day.

A second group of patients was similarly studied. All of these subjects were observed in the immediate postoperative period or during convalescence from a variety of surgical procedures. It was found that immediately following surgical trauma when nitrogen loss was greatest, there was also the most marked loss of riboflavin through urinary excretion. In one patient, following a major surgical procedure, 3.2 mg. of riboflavin were excreted in the first five days of the postoperative period. In all these patients, the quantity of riboflavin retained increased as nitrogen equilibrium was approached and positive nitrogen balance was achieved. Certain of the patients were in poor states of nutrition prior to study. These patients were found to retain most of the riboflavin administered when caloric and protein intakes were increased to allow for positive nitrogen balance and gain in body weight. After a further period of high level intake of protein and riboflavin, these patients excreted most of the administered riboflavin, indicating saturation of tissues with the vitamin.

Patients with diabetes mellitus were selected for a third group of subjects. The decision was made to investigate this type of patient because of the relative ease of manipulation of nitrogen balance. Withdrawal of insulin will result in immediate disturbance of carbohydrate metabolism and negative nitrogen balance. Therefore, attempts were made to vary nitrogen balance at will by graded withdrawal or administration of insulin. The intake of riboflavin was maintained at 2 mg. per day. The negative nitrogen balance induced by reduction in dosage of insulin was usually associated with decreased retention of riboflavin. In some instances the urinary excretion of the vitamin exceeded the intake. By providing additional protein in the diet plus insulin sufficient to prevent negative nitrogen balance, it was possible to determine the effect of glycosuria and polyuria on riboflavin excretion or retention. Despite polyuria and loss of as much as 50 per cent of the ingested carbohydrate in the urine, riboflavin retention was good, and less than 50 per cent of the vitamin administered was excreted so long as patients were maintained in positive nitrogen balance. In other patients it was impossible to induce negative nitrogen balance despite withdrawal of insulin and the resultant excretion of large quantities of carbohydrate in the urine. Under these circumstances most of the ingested riboflavin was retained. Four obese diabetic patients consumed a diet providing calories below maintenance levels. These patients were consistently in negative nitrogen balance and almost uniformly excreted most of the riboflavin ingested in the diet.

The observations in the three groups of patients included in this investigation are consistent in demonstrating a direct relation-

ship between nitrogen and riboflavin retention when levels of riboflavin intake are adequate but not excessive. Administration of larger quantities of riboflavin may result in excretion of most of that administered even with patients in positive nitrogen balance if saturation of tissues and body stores has been accomplished. Since riboflavin occurs in its free form in but a small proportion of the total body riboflavin, most of the vitamin in tissues must be considered to be bound with protein. In a phase of protein breakdown such as that in the period immediately following surgical trauma when patients are in negative nitrogen balance and excrete more than the quantity of riboflavin administered, it can be presumed that part of the protein degraded is that of the flavoprotein complex. This would seem to indicate that the flavoproteins exist as a component in a larger body pool of labile proteins.

Quite another condition exists when patients are in negative nitrogen balance associated with inadequate intake of calories, or because of withdrawal of insulin in diabetics. In this instance riboflavin is poorly retained but is not excreted in quantities which exceed those ingested. Pollack and Bookman (*loc. cit.*) ascribe this either to failure of synthesis of flavoproteins or to increased breakdown of existing proteins with synthesis remaining normal.

The findings described pose a question concerning the validity of attempts to assess the nutritional status of an individual relative to riboflavin by determinations of rates of urinary excretion, since this is shown to relate directly to nitrogen balance in or just preceding the period of study.

These investigations demonstrate the interdependence of protein and riboflavin metabolism in man. Also, they indirectly describe the flavoproteins as one component of a larger pool of quite labile body proteins.

WARTIME STARVATION IN GREECE

The severe famine in Greece (1941 to 1943) has not been covered in a monograph and even the journal articles are scarce. V. G. Valaoras (*Milbank Memorial Fund Quart.* **24,** *215 (1946)*; *Nutrition Reviews* **5,** *28 (1947)*) presented vital statistics for the famine period, indicating that the starvation mortality of adult males was higher than that of women and children. In Athens the number of "hunger" deaths rose sharply during November 1941, reaching in December a value of 400 deaths per week and decreasing to about 200 by April 1942. He presented also body measurements (height and weight) made on children.

Effects of the famine were considered from the clinical point of view by T. Doxiadès (*Rev. méd. de la suisse rom.* **70,** *224 (1950)*). Even before the war Greece had to import food from abroad, and the intake of animal protein was low. With the occupation of Greece in the spring of 1941, all imports of food were stopped. To make conditions worse, the country was divided into three zones of occupation (German, Italian, and Bulgarian), with the lines of demarkation based on political and military rather than economic considerations. There were difficulties in shipping food—the little that was left after confiscations by the occupation authorities—from one zone to the other and from the country to the cities. In March of 1942 the International Red Cross began to provide food supplements, increasing from about 350 calories per man per day to about 700 calories in November 1942. The official Greek rations were very meager (below 500 calories; in November 1941 and February 1942 about 200 calories). The rest had to be obtained largely from the black market.

The effects of prolonged severe under-

nutrition resembled in almost every detail the all-too-familiar pattern observed elsewhere in war-torn Europe and on many occasions throughout history. Doxiadès described these effects as he saw them in the streets, in his private office, and in the hospital. The people, whose morale had suffered as a result of the military defeat, became more depressed and apathetic. Nobody seemed to be in a hurry. Patients began to come in with complaints of general fatigue. At first these patients were predominantly men. They lost 5 to 10 kg. of body weight while the weight loss of women was 1 to 2 kg. The author attributes this difference to the fact that the men had to walk considerable distances because the public transportation system became ineffective, while the women could be largely spared this energy expenditure. Money was losing its value. The beggars, whose numbers increased markedly, refused money: they wanted something to eat.

New starvation symptoms were added as summer passed into fall: emaciation, giddiness, nocturia. In men the loss of weight reached 12 to 20 kg., in women 8 to 10 kg. People became indifferent to all but matters of finding food. Hospitals became jammed with individuals grey-pale in appearance, with slowing of pulse rate (bradycardia), relatively low blood pressure, some edema which could not be explained on cardiovascular or renal basis, low blood sugar and low nonprotein nitrogen in the blood. In the streets one daily saw people stagger and fall from exhaustion, and nobody cared. Removal of the dead bodies from the streets and parks was the only remaining active public service. The streets became cluttered because there was nobody to remove the refuse. Famished children could be seen digging in garbage cans in their search for something to eat.

The clinical effects of famine were described under three categories: acute starvation (with a "wet" and "dry" form), prolonged undernutrition, and avitaminoses. The story of the vitamin deficiencies is short. There was little evidence of acute deficiency of vitamins A, C, and of thiamine. Effective distribution of cod liver oil is credited with the prevention of rickets. During the winter of 1941–1942 a large number of people suffered from chilblains; the author considers the possibility that this might have been a symptom of vitamin A deficiency. The only bona fide vitamin deficiency disease was pellagra, which affected thousands of persons in the spring of 1942.

One of the classic effects of profound undernutrition on public health is the rise in the incidence of tuberculosis. In Greece, in comparison with the experience in western Europe, this rise was unexpectedly small, with the increase in morbidity estimated at about 10 per cent. The author comments that this difference may be due to climatic factors. Other diseases of the respiratory apparatus were not affected. The effects on the cardiovascular system were actually favorable. Hypertension, and also diabetes, disappeared almost completely. On the other hand, there was an increase in the incidence of gastroduodenal ulcers and of colitis. The hydrochloric acid content of the gastric juice was often low. The number of cases of leukemia and of pernicious anemia, both otherwise rare in Greece, increased. The activity of organs of internal secretion was reduced. The rise in the occurrence of Addison's disease indicated hypofunction of the suprarenal glands. Reduced activity of the thyroid resulted in the disappearance of hyperthyroidism and the increase of cases of hypothyroidism (myxedema). The menstrual cycle was obliterated in the majority of women and reappeared spontaneously when the nutritional situation improved. In men the libido disappeared, to return only after a prolonged period of refeeding.

Considerable space was devoted to the clinical and pathologic aspects of severe starvation. The quantitative data were mostly limited to averages. Of interest are the values for the loss of weight of internal

organs, observed in individuals who lost 30 to 60 per cent of the initial gross body weight. For the heart the decrement is indicated as 25 to 50 per cent. For the brain the decrement is larger than cited by other authors (10 to 20 per cent). Among 1347 autopsies carried out between November 1941 and March 1942, "starvation" (*maladie de famine*) was indicated as the principal cause of death in 67 per cent of the cases. Bronchopneumonia was noted as a complicating factor in 20 per cent and tuberculosis in 5 per cent of the cases.

Doxiadès' material was originally presented in the form of a lecture before a medical audience at Lausanne, Switzerland, which necessarily narrowed the scope of the presentation. However, the contribution made by the paper to the general fund of knowledge concerning the effects of starvation is very modest, and even a more extensive presentation of the material—limited by the conditions under which it was collected—would not likely prove more revealing. There are no data on nutritional rehabilitation.

NOTES

Letter to the Editor

Dear Sir:

Although the review on Caloric Priorities in Parenteral Nutrition by T. B. Van Itallie (*Nutrition Reviews* **9,** *193 (1951)*) summarizes very well the need for and advantages of intravenous fat, still unanswered is the question most clinicians ask—what is the optimum proportion of caloric to protein intake when less than a full quota of each is temporarily inevitable? Practical limitations, at the present time at least, almost compel less than a full intake when the parenteral route is necessary. However, there are other clinical situations in which a full nutritional intake is temporarily impossible or undesirable. A well-known example was the experience with the victims of concentration camps during the last war. Many of these unfortunates could not safely ingest a full food intake so that their survival depended upon devising a limited ration as the initial diet. Even now, severely malnourished patients are seen too frequently in general hospital practice. They too can digest and absorb only a limited amount of food in the beginning. While in these cases calories and protein are both necessary, there is still considerable need for further data as to the optimal proportion of energy-providing and protein-rebuilding foods—a proportion which during the critical period of therapy will best reduce mortality and lead to the most rapid physiologic rehabilitation.

Regardless of the reasons for the need for a temporarily limited intake, the best combination of calories and protein is one which leads to the highest level of positive nitrogen balance. Should it be impossible for some reason to achieve nitrogen balance immediately, it is just as obvious that the best diet is one which leads to the lowest level of negative nitrogen balance.

It is in surgical patients temporarily unable to eat that the question is most often asked regarding the relative need for calories and protein. The basic routine in these patients is usually 2 or 3 liters of 5 per cent glucose, *i.e.*, 100 to 150 g. of glucose per day.

Although the addition of 100 g. of an amino acid mixture (5 per cent in 5 per cent glucose) may not lead to nitrogen balance, the degree of negative nitrogen balance can be and often is considerably reduced thereby. This is not true of the addition of 100 g. of glucose (as 10 per cent glucose). For example, J. L. Gamble (*Harvey Lectures* **42,** *247 (1946–47)*) found no change in the negative nitrogen balance when the daily caloric intake was increased from 100 g. of glucose to 200 g. of glucose in normal humans. The same finding was described by the writer and his co-workers (R. Elman *et al.*, *Ann. Surg.* **130,** *703 (1949)*) in a series of postoperative patients given the same amounts of glucose intravenously. It would seem therefore that adding amino acids to the basic intake of 5 per cent glucose (rather than further glucose) has the advantage of sparing tissue protein loss as well as carrying out a nutritional principle of giving as many as possible of the essential nutritional elements even though a limited amount may be temporarily possible.

One of the puzzling features of parenteral nutrition in postoperative patients is the wide variation from case to case observed in the degree of nitrogen balance obtained with intravenous amino acid mixtures, especially when the intake is subcaloric. These variations must be due among other things to differences in the ability of patients to utilize their adipose tissue for energy, especially in patients who are well supplied with body fat. For the short periods in which parenteral feeding is ordinarily necessary, means devised to enable the body to utilize more of its adipose tissue might be of nutritional advantage in achieving better nitrogen balance, at least

until an acceptable preparation of intravenous fat becomes available.

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Pathology of Nutritional Diseases

Clinical medicine for generations was based largely on the study of anatomic and histopathologic material. In recent times the physiologic and biochemical approach has supplemented this morphologic background. The intensive interest and progress in diseases of nutrition has been largely a development of the physiologic and biochemical age. Advancement in the understanding of these disturbances was so great that little attention was paid to the lack of morphologic data. Nutrition as a branch of medicine has achieved a permanent place. To complete this maturation of the new science it is important to study the histopathology of the nutritional disturbances. To this end a Registry of Pathology of Nutritional Diseases has been founded. This Registry is sponsored by the American Institute of Nutrition.

The Registry of Pathology of Nutritional Diseases is a unit of the American Registry of Pathology of the Armed Forces Institute of Pathology in Washington, D. C., and is under the auspices of the Committee on Pathology of the National Research Council.

The purposes of the Registry are to provide for the accumulation and maintenance of collections of pathologic materials and related case records in such special fields of nutritional diseases in which it is anticipated that unknown diagnostic and prognostic criteria as well as evaluations of various methods of treatment can be discovered and established through morphologic studies.

The Registry will provide consultation service in the field of nutritional pathology and prepare teaching material in such form as may be most readily and satisfactorily available for loan to qualified students and institutions.

The cooperation of all individuals and institutions is invited. The registry has been authorized to receive any documented material sent by physicians, hospitals, laboratories, or other reliable sources. For further information write to Dr. Herbert Pollack, 70 East 77th Street, New York City, or to the Registry for Nutritional Pathology, Armed Forces Insititute of Pathology, Washington 25, D. C.

Plastic Tubing for Intravenous Feeding

Prolonged parenteral feeding is often made extremely difficult by the sclerosing effect upon veins of the solutions used, and by the difficulty of maintaining the needle in place. Plastic tubing has been used for this purpose, and in the experience of one investigator has been extremely useful, not only for feeding, but for the administration of blood plasma and similar substances. These results are described by C. D. Bonner (*New Engl. J. Med.* **245,** *97 (1951)*). A total of approximately 850 l. of various fluids were administered to 23 patients, and the author estimates that about 450 venipunctures were avoided. The patients were able to get in and out of bed with the tubing in place.

Recent Nutrition Books

Studies of Undernutrition, Wuppertal 1946–9. By Members of the Department of Experimental Medicine, Cambridge, and associated workers. His Majesty's Stationery Office, London, 1951. Pp. 404. Price: $2.85. Copies may be obtained from British Information Services, 30 Rockefeller Plaza, New York 20, N. Y.

Biological Antagonism. The Theory of Biological Relativity. Gustav J. Martin. The Blakiston Company, New York, 1951. Pp. 516.